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Comparison of a *coq7* deletion mutant with other respiration-defective mutants in fission yeast

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Abbreviations: PHB, *p*-hydroxybenzoate; PM, pombe minimum; Q, ubiquinone.

ABSTRACT

Among the steps in ubiquinone (Q) biosynthesis, that catalyzed by the product of the *clk-1/coq7* gene has received considerable attention because of its relevance to life span in *C. elegans*. We analyzed the *coq7* ortholog (denoted *coq7*) in *Schizosaccharomyces pombe,* to determine whether the *coq7* gene has specific roles that differ from those of other *coq* genes. We first confirmed that *coq7* is necessary for the penultimate step in ubiquinone biosynthesis, from the observation that the deletion mutant accumulated the ubiquinone precursor demethoxy ubiquinone (DMQ)-10 instead of Q-10. The *coq7* mutant displayed phenotypes characteristic of other ubiquinone-deficient *S. pombe* mutants, namely, hypersensitivity to hydrogen peroxide, a requirement for antioxidants for growth on minimal medium, and an elevated production of sulfide. To compare these phenotypes with those of other respiration-deficient mutants, we constructed cytochrome *c* (*cyc1)* and *coq3* deletion mutants. We also assessed accumulation of oxidative stress in various ubiquinone-deficient strains and in the *cyc1* mutant by measuring mRNA levels of stress-inducible genes and the phosphorylation level of the Spc1 MAP kinase. Induction of *ctt1* encoding catalase and the *apt1* gene encoding a 25kd protein, but not that of *gpx1*, encoding glutathione peroxidase, was indistinguishable in four ubiquinone-deficient mutants, indicating that the oxidative stress response is at similar levels in the tested strains. One new phenotype was observed, namely, loss of viability in stationary phase in both the ubiquinone-deficient mutant and in the *cyc1* mutant. Finally, Coq7 was found to localize in mitochondria, consistent with the possibility that ubiquinone biosynthesis occurs in mitochondria in yeasts. In summary, our results indicate that *coq7* is required for ubiquinone biosynthesis and the *coq7* mutant is not distinguishable from other ubiquinone-deficient mutants, except that its phenotypes are more pronounced than those of the *cyc1* mutant.

INTRODUCTION

Ubiquinone (or Coenzyme Q) is essential for aerobic growth and for oxidative phosphorylation because of its known role in electron transport. Recently, however, multiple additional functions for ubiquinone have been proposed. One such function is its apparent role as a lipid-soluble antioxidant that prevents oxidative damage to lipids due to peroxidation [1]. Studies using ubiquinone-deficient yeast mutants support an *in vivo* antioxidant function [2, 3]. Other studies have proposed a role linking ubiquinone to sulfide metabolism through sulfide-ubiquinone oxidoreductase in fission yeast, but not in budding yeast [4, 5]. In addition, an elegant study showed that ubiquinone (or menaquinone) accepts electrons generated by protein disulfide formation in *Escherichia coli* [6].

The ubiquinone biosynthetic pathway comprises ten steps, including methylations, decarboxylations, hydroxylations, and isoprenoid synthesis and its transfer. The elucidation of this pathway has mostly involved studying respiratory-deficient mutants of *E. coli* and *Saccharomyces cerevisiae* [7, 8]. The length of the isoprenoid side chain of ubiquinone varies among organisms. For example, *S. cerevisiae* has Q-6, *E. coli* has Q-8, rats and *Arabidopsis thaliana* have Q-9, and humans and *Schizosaccharomyce pombe* have Q-10 [8-10]. The length of the side chain is determined by polyprenyl diphosphate synthase [11, 12], but not by 4-hydroxybenzoate-polyprenyl-diphosphate transferases, which catalyze the condensation of 4-hydroxybenzoate and polyprenyl diphosphate [13, 14]. Typically, Q-10 can be synthesized by expression of decaprenyl diphosphate synthase from *Gluconobacter suboxydans* in *E. coli*, yeast and rice [15, 16]. A different type of ubiquinone (varying from Q-6 to Q-10) does not affect the survival of *S. cerevisiae* [17, 18] or *E. coli* [17, 19]. Recently, however, it was shown that the various ubiquinones do have type-specific biological effects, as exogenous Q-7 was not as efficient as Q-9 in restoring growth of the *Caenorhabditis elegans* Q- less mutant [20].

The *clk-1* mutant *of C. elegans*, which accumulated the precursor DMQ, due to lack

of the penultimate step in ubiquione biosynthesis was reported to exhibit a prolonged life-span, developmental delay and reduction in brood size [21]. The *clk-1* gene in *C. elegans* is a functional orthlog of *COQ7*, which was found to encode DMQ monooxygenase in *S. cerevisiae* [22]. *E. coli* UbiF also catalyzes the same step as COQ7 and Clk-1, based on the observation that *clk-1* rescues Q biosynthesis in an *E. coli ubiF* mutant [23]. *COQ7* orthologs are also recognized in mammals [24]. A *clk-1* homozygous mutant mouse exhibits embryonic lethality [25], but interestingly, a heterozygous *clk-1* mutant has an extended life span [26, 27]. Thus, Coq7, Clk-1 and UbiF are highly conserved proteins in different kingdoms, but intriguingly, no apparent ortholog has yet been described in plants as judged from DNA sequence analysis [8].

The long life span of the *C. elegans clk-1* mutant has been attributed to the presence of DMQ-9, because it is believed to retain fewer pro-oxidant properties than Q, and has been shown to retain partial function in the respiratory chain [28]. However, Q-8 from *E. coli* and endogenous RQ-9 have also been shown to influence the life extension phenotype in the *clk-1* mutant [29, 30]. Thus, the physiologic contributions of multiple types of quinones should be considered when attempting to account for the long life span of the *C. elegans clk-1* mutant. However, because of the complexity of quinone function, it has not been possible to determine which specific quinone plays the most important role in the long life span phenotype. *S. pombe* provides an excellent model system in which to determine whether DMQ has a specific biological role because no exogenous nor endogenous quinone other than Q-10 is present in this species.

Our group has so far identified four genes related to ubiquinone biosynthesis in *S. pombe*. Two genes (*dps1* and *dlp1*) together encode a hetero-tetrameric decaprenyl diphosphate synthase [3, 5] which is responsible for synthesis of the isoprenoid side chain of ubiquinone. The third (*ppt1*) encodes PHB polyprenyl diphosphate transferase involved in transfer of the side chain to PHB. The fourth is *coq8* [31], for

which a function has not yet been ascribed, but which is essential for ubiquinone biosynthesis.

In the present study, we characterized the *S. pombe coq7* gene and compared a *coq7*-deficient mutant to other respiration-deficient mutants, namely, a *coq3* mutant lacking a putative *O*-methyl transferase and a *cyc1* mutant lacking cytochrome *c*. Because *clk-1* in *C. elegans* has been the focus of much recent research, we first assessed phenotypic differences between the *coq7* mutant and other ubiquinone-deficient mutants. A *coq7* disruption mutant was found not to produce Q-10, but accumulated the precursor DMQ-10. Even though the *coq7* mutant accumulated the precursor, its phenotypes were indistinguishable from those of other ubiquinone-deficient *S. pombe* mutants, which argues against a possible role for DMQ in respiration.

Results

Cloning of the *coq7* gene and construction of a *coq7* deletion mutant.

While it has been reported that a precursor of ubiquinone (DMQ) is relevant to the life extension phenotype in the *C. elegans clk-1/coq7* mutant [32], DMQ accumulation in an *S. cerevisiae coq7* mutant (not a deletion allele) was found not to play a role in electron transfer [33]. We next sought to determine whether the *S. pombe coq7* deletion mutant accumulated a precursor and displayed any specific phenotypes. A putative gene for demethoxy ubiquinone hydroxylase has been reported in the *S. pombe* genome by the Sanger center (http://www.genedb.org/genedb/pombe/). This gene (SPBC337.15c) shows high sequence similarity with the *COQ7* gene from *S. cerevisiae* and is hereafter denoted *coq7*. *S. pombe* Coq7 is 45% and 41% identical at the amino acid level with *S. cerevisiae* Coq7 and *C. elegans* Clk-1, respectively (Fig. 1).

To investigate the function of the fission yeast *coq7/clk-1* gene, we first generated a *coq7*-deficient fission yeast mutant by homologous recombination. To this end, we

first amplified the *coq7* gene from *S. pombe* genomic DNA by PCR to yield a 2.2-kb DNA fragment containing *coq7* gene and flanking DNA. We next constructed the plasmid pBUM7, in which the *coq7* gene was disrupted by the *ura4* gene (Fig. 2A). This plasmid was then made linear by appropriate restriction digestions and was used to make a *coq7* deletion mutant named LN902 (Δ*coq7*) from the *S. pombe* wild-type diploid strain SP826 (Fig. 2B). Genomic DNAs from wild type and LN902 (Δ*coq7*) were analyzed by Southern hybridization to confirm the disruption of *coq7* by *ura4* (Fig. 2C and Experimental Procedures).

LN902 accumulates a quinone-like intermediate instead of ubiquinone.

To determine whether LN902 (Δ*coq7*) produced ubiquinone or not, lipid extracts were prepared from wild-type SP870 and LN902 and analyzed by reverse-phase HPLC. The extracts from SP870 yielded a major peak at 20.4 min (not shown), which is consistent with authentic Q-10, while the extracts from LN902 failed to yield this peak, but instead, yielded a new peak at 19.9 min. This peak was close to, but apparently eluted faster than that of authentic Q-10, as the mixture of both authentic Q-10 and extracts from LN902 (Δ*coq7*) yielded two separable peaks (Fig. 3A).

The identification of the main quinone-like compound isolated from LN902 and authentic Q-10 was performed by EI mass spectrometry. EI mass spectrometry of authentic Q-10 and the quinone-like compound from LN902 produced signals at *m/z* 863 and 833, respectively. The quinone-like compound from LN902 yielded a protonated molecular ion corresponding to that of DMQ-10 (calculated mass is 832.28 Da, Fig. 3). This result is consistent with a defect in the penultimate step of ubiquinone biosynthesis in LN902 and provides evidence that *coq7* in fact encodes demethoxy ubiquinone hydroxylase. Thus, the *S. pombe coq7* disruptant accumulated the ubiquinone precursor DMQ like the *C. elegans clk-1* mutant [32], and unlike the *S. cerevisiae coq7* deletion mutant [33].

Complementation of coq7 disruptant mutant with S. cerevisiae COQ7.

To test for functional conservation between *S. cerevisiae* Coq7 and *S. pombe* Coq7, LN902 (Δ*coq7*) was transformed with plasmids pREP1-coq7Sp and pREP1-COQ7, containing only the *S. pombe coq7* gene or the *S. cerevisiae COQ7* gene, respectively, both expressed under the control of the strong promoter *nmt1*. LN902 transformants harboring either pREP1-coq7Sp or pREP1-COQ7 were then plated on PM minimum medium. After a few days of incubation, LN902 harboring only the pREP1 vector or pREP1-COQ7 formed very tiny colonies, while LN902 harboring pREP1-coq7Sp grew as well as the wild-type strain. Thus, *coq7* on the plasmid rescued the *coq7* disruptant, but expression of *S. cerevisiae COQ7* was unable to complement the LN902 mutant. Because the N-terminal sequence of COQ7 is exceptionally long relative to other Coq7 sequences (Fig. 1), we speculated that the COQ7 signal sequence did not function properly in *S. pombe*. Consequently, we constructed pREP1-TPCOQ7, which contains the entire *COQ7* gene fused with a putative mitochondrial transit peptide (TP) from the *ppt1*$^{+}$ gene [14], anticipating that the *S. pombe* signal sequence for mitochondrial transfer would be required for Coq7 function. An LN902 transformant harboring pREP1-TPCOQ7 was found to grow better than LN902 harboring only the pREP1 vector (Fig. 4A). Ubiquinone was subsequently extracted from each strain (Fig. 4B). Q-10 was detected in the wild-type strain, in LN902 harboring pREP1-coq7Sp, and in LN902 harboring pREP1-TPCOQ7, while DMQ-10 was only detected in LN902 harboring the pREP1 vector. A small amount of Q-10 were detected in LN902 harboring pREP1-TPCOQ7. Thus, pREP1-TPCOQ7 partially complements the *coq7* disruptant and allows production of a small amount of Q-10 in *S. pombe*. This result also indicates that a small amount of Q-10 is sufficient for growth. While perfect complementation was not observed, we conclude that *S. pombe* Coq7 and *S. cerevisiae* COQ7 are functional orthologs.

Construction of *cyc1* and *coq3* deletion mutants.

To compare the *coq7* deletion mutant to other respiration-deficient mutants, we constructed deletion mutants of *cyc1* encoding cytochrome *c* [34] and *coq3* encoding a putative *O*-methyl transferase involved in ubiquinone biosythesis. To our knowledge, deletion mutants defective in electron transfer in fission yeast other than ubiquinone-deficient mutants, have not been reported. We speculate that this *cyc1* deletion mutant may be representative of a typical respiration-deficient mutant in *S. pombe*. Deletion mutants of *cyc1* and *coq3* were constructed similarly using a two-step PCR method based on a *kanMX6* module [35] as described in Materials and Methods (Fig. 2). Using the *kanMX6* module, a *cyc1::kanMX6* fragment was constructed and used to disrupt the chromosomal *cyc1* allele in the haploid wild-type PR110 strain. The disruption was verified by PCR using appropriate primers. To obtain the mutants in the same genetic background, the *coq7* deletion mutant was constructed using the *kanMX6* module, and the resulting strain was designated RM1 (*coq7*:: *Km*^{r}). The disruption was confirmed by Southern blotting.

Respiration deficiency of Δ*cyc1,* Δ*coq7* and Δ*coq3* mutants.

To confirm that the constructed Δ*cyc1,* Δ*coq7* and Δ*coq3* mutants were in fact respiration-deficient, oxygen consumption was measured during growth. The Δ*cyc1*, Δ*coq7* and Δ*coq3* mutants were found to consume oxygen at about 3-9% of the rate of the wild type strain. Because oxygen-consuming reactions unrelated to respiration are known, the rate was not expected to decrease to zero. As further confirmation of a defect in respiration, the mutants were grown on a plate containing TTC (2,3,4-triphenyltetrazolium chloride) and colony color was scored [36]. If respiration is normal, TTC turns red, but if not, the colonies remain white. Colonies of the three mutants Δ*cyc1*, Δ*coq7* and Δ*coq3* were found to be white, whereas those of the wild type parent turned red, as expected (data not shown).

Phenotypes of the coq7 disruptant and other respiration-deficient

mutants.

We previously reported that KS10 (*Δdps1::ura4*), RS312 (*Δdlp1::ura4*), NU609 (*Δppt1::ura4*), and NBp17 (*Δcoq8*) which are disrupted in *dps1* (one component of decaprenyl diphosphate synthase), *dlp1* (another component of decaprenyl diphosphate synthase), *ppt1* (PHB-polyprenyl diphosphate), and *coq8* (an essential gene for ubiquinone biosynthesis), respectively, are unable to produce ubiquinone and have other notable phenotypes [31], including sensitivity to H_2O_2 and Cu^{2+}, and a growth requirement for cysteine or glutathione on minimal medium. RM1(Δ*coq7*) was also tested for these phenotypes. RM1 was first grown on PM-based medium with and without 200 μg/ml of added cysteine. The addition of cysteine effectively restored growth to wild type levels as observed for the *ppt1* disruptant [14] when treated similarly (data not shown). Our previous reports suggest that all ubiquinone-deficient strains are sensitive to oxygen radical producers [5, 14]. Here, we found that the growth of RM1 (Δ*coq7*), RM2 (Δ*coq3*) and RM3 (Δ*cyc1*) was severely inhibited by the presence of 0.5 mM H_2O_2 (Fig. 5). Both RM1 (Δ*coq7*) and RM2 (Δ*coq3*) were inhibited by 1.5 mM Cu^{2+}, but not RM3 (Δ*cyc1*) (Fig. 5). The oxidants at these concentrations did not affect the growth of wild-type cells (Fig. 5). These results are consistent with previous results [5, 14]. Unlike the ubiquinone-deficient mutants, the Δ*cyc1* mutant was not affected by 1.5 mM Cu^{2+}, which will distinguish the ubiquinone-deficient mutants and a respiration deficient mutant (see Discussion).

Ubiquinone and the oxidative stress response.

From the above results, we expected that several genes induced by oxidative stress would be highly expressed in ubiquinone-deficient strains. Thus, we tested the induction of three genes: *ctt1*$^+$ encoding catalase, *gpx1*$^+$ encoding glutathione peroxidase and *apt1,* which is known to be induced under conditions of oxidative stress through the Pap1 transcription factor [37]. It is known that induction of *apt1* and *gpx1* genes depend solely on the Pap1 and Atf1 transcription factors, respectively,

and that induction of *ctt1* gene is dependent on both Pap1 and Atf1 in *S. pombe* [38]. While induction of *ctt1*$^+$ and *apt1* occurred in all ubiquinone-deficient strains, induction of *gpx1*$^+$ was not observed in any of the tested strains (Fig. 6). However, in the wild-type strain treated with 1 mM H_2O_2 for 15 min, a high level of induction of *ctt1* and *gpx1*, but not *apt1*, was observed as previously reported [38, 39]. Higher levels of H_2O_2 have been reported to induce *ctt1*$^+$ through Atf1, while lower levels induce *ctt1* and *apt1* through Pap1[38, 39]. Consistent with the observation that these genes are under the control of Spc1, only low levels of transcripts were observed in a *spc1* mutant. (Fig. 6). Our results indicate that at low levels of H_2O_2, the ubiquinone-deficient mutants accumulate oxidative stress in proportion to the H_2O_2 dose. Furthermore, it appears that in Q-deficient fission yeast, the Pap1 pathway is functional.

Phosphorylation of Spc1 MAP kinase.

To further assess the physiologic consequences of oxidative stress in cells, we measured the phosphorylation status of the Spc1 MAP kinase. Because oxidative stress is transduced into the cells by the stress-responsive MAP kinase cascade, the phosphorylation status of Spc1 MAP kinase should be one sensitive indicator of oxidative stress. When we measured the phosphorylation status of Spc1 by a phospho-specific antibody, we found that Spc1 in both the Δ*cyc1* and Δ*coq7* mutants was phosphorylated. Phosphorylation of Spc1 was not observed in wild type cells in the absence of H_2O_2, nor in a mutant of *sir1* that encodes sulfite reductase or in a mutant of *hmt2* that encodes sulfide-ubiquinone oxidoreductase. Thus, combined with the above results, evidence for oxidative stress was clearly observed in the Δ*coq7* and Δ*coq3* mutants (Fig. 7).

Production of hydrogen sulfide in *S. pombe* mutants.

We found that when *S. pombe* strains disrupted for *ppt1*, *dps1* or *dlp1* were grown,

they produced an aroma of rotten-eggs, reminiscent of hydrogen sulfide. Indeed, production of H_2S was positive when assayed with lead acetate, leading to formation of PbS. Strains deficient in ubiquinone produced H_2S, but wild type cells did not. We measured the amount of acid-labile sulfide present in cells during growth and found that RM1 (Δ*coq7*) and RM2 (Δ*coq3*) produced a maximum amount of about 8 to 20 times more S^{2-} than wild-type (Fig. 8). This is consistent with results obtained with other ubiquinone-deficient mutants [5, 31]. At the same time, JV5 (Δ*hmt2*) and RM3 (Δ*cyc1*) were found to produce less sulfide. While the *hmt2* deletion mutant was known to produce sulfide [40], to our knowledge, this is the first observation that a respiration-deficient mutant such as RM3 produces slightly more sulfide than wild type, but less than ubiquinone-deficient mutants. Because the *sir1* gene encodes sulfite reductase that catalyzes production of sulfide from sulfite, we confirmed that a *sir1* mutant did not produce any detectable sulfide (Fig. 8). We also found that the maximum production of sulfide differed among tested strains and was also highly sensitive to growth conditions, perhaps due in part to its volatility. Thus, careful measurement will be required to properly assess this phenotype. These results suggest that ubiquinone is an important factor in sulfide oxidation in *S. pombe*.

Loss of viability at stationary phase.

We reasoned that if damage due to oxidative stress accumulates, this might be evidenced by a reduction in viability in damaged Δ*coq7* cells following prolonged incubation. To test this, the PR110, RM1 (Δ*coq7*), RM2 (Δ*coq3*), RM3(Δ*cyc1*), and JZ858 (Δ*cgs1*) strains were incubated in liquid PMA medium at 30˚C until reaching a density of 1.0 x10^7 cells/ml. Cells were further incubated an additional 4 days and assessed for survival. The viability of the Δ*coq7,* Δ*coq3 and* Δ*cyc1* cells decreased rapidly (Fig. 9), that of the Δ*cgs1* cells less so, while that of wild type did not. Because *cgs1* that encodes the regulatory subunit of A-kinase is known to be necessary for viability during stationary phase, a *cgs1* mutant was used as a negative control [41]. No differences in

survival among the ubiquinone-deficient mutants and the *cyc1* mutant were observed. We conclude that respiratory function is necessary for survival during stationary phase.

Mitochondrial localization of the Coq7 protein.

Because the ubiquinone biosynthetic enzymes are localized in the mitochondria of *S. cerevisiae* [42], and Ppt1 has been shown to localize in mitochondria in *S. pombe* [14], we expected that ubiquinone biosynthesis would also occur in the mitochondria in *S. pombe.* Localization of Coq7 in *S. pombe* was examined by constructing a Coq7-green fluorescent protein (GFP) fusion. While GFP alone localized in the cytoplasm, the Coq7-GFP fusion protein localized in mitochondria (Fig. 10). Thus, to our knowledge, Coq7 appears to be the second ubiquinone biosynthetic enzyme shown to be located in mitochondria in *S. pombe*.

Discussion

In this study, we attempted to answer two major questions: 1) does DMQ-10 (an intermediate compound in ubiquinone biosynthesis) have specific functions in fission yeast; and 2) do ubiquinone-deficient mutants differ from other respiration-deficient mutants in fission yeast? Our answer to the first question was negative, but the answer to the second was positive.

We first showed that Coq7 catalyzes the penultimate step in ubiquinone biosynthesis. Unlike the corresponding *S. cerevisiae* mutant, the precursor demethoxy ubiquinone (DMQ)-10 accumulated in the *S. pombe coq7* deletion mutant as observed in the *C. elegans clk-1* null mutant and in mouse *clk-1* knock out cells [25, 32]. Despite the accumulation of DMQ-10, the phenotype of the *coq7* mutant is indistinguishable from that of other *S. pombe coq* deletion mutants, which suggests that DMQ is not an electron acceptor in respiring *S. pombe* cells as reported for *S. cerevisiae* [33], but is partially functional in respiration in *C. elegans* and mouse [25, 28]. Our result does not

support the proposal that DMQ plays a role in electron transfer. Nonetheless, our results must be interpreted cautiously, because species-specific differences in function may exist between yeasts, *C. elegans,* and mouse. One such difference can be found in the first step of the electron transfer system. Complex I plays a role in NADH oxidation in animals including *C. elegans,* but in yeasts, NADH-ubiquinone reductase functions instead [43]. These differences in the two enzymes may have consequences for DMQ function, as the binding sites of quinones are present in complex I, but it is not clear if they are present in the NADH-ubiquinone reductase of yeasts.

S. cerevisiae COQ7 can only partially complement an *S. pombe* Δ*coq7* mutant. One explanation may be insufficient transport of ScCoq7 into the *S. pombe* mitochondria. Alternatively, a functional ubiquinone-enzyme complex may not form. Such a complex has been proposed to exist in *S. cerevisiae* [44] and may also exist in *S. pombe.* However, no direct evidence presently supports the existence of such a complex in *S. pombe*.

Coq7 localized in mitochondria with other *S. pombe* Coq proteins as well (our unpublished data). Because the Coq components in *S. cerevisiae* have been shown to localize in the inner membrane [44], it is certain that biosynthesis of CoQ occurs in mitochondria in these two yeasts. However, it was recently shown that one of the prenyl diphosphate synthases in *A. thaliana* localizes in the ER [45] while the PHB prenyl diphosphate transferse (AtPpt1) localizes in mitochondria [46]. This difference illustrates the diversity of enzyme localization in different organisms.

Monitoring oxidative stress by measuring expression levels of *ctt1, apt1,* and *gpx1* and also by Spc1 phophorylation clearly showed that ubiquinone-deficient mutants and a cytochrome *c* mutant are stressed. These are sensitive methods to monitor intracellular oxidative conditions. Use of these endpoints indicated that without a properly functioning electron transfer system, cells become stressed, resulting in activation of the stress-sensitive MAP kinase, and increasing expression of downstream target genes such as *ctt1* and *apt1*. These results are consistent with a previous report

that ubiquinone-deficient mutants are sensitive to exogenous hydrogen peroxide [14]. Comparison of the ubiquinone-deficient mutants with the cytochrome *c* mutant in fission yeast indicated a general similarity in phenotypes, but with some less pronounced in the latter mutant. The cytochrome *c* mutant was not as sensitive to Cu^{2+} nor produced as much as sulfide as the ubiquinone-deficient mutants. These results may reflect differences in a requirement for ubiquinone in reactions unrelated to respiration. Sulfide accumulated to high levels in all the tested ubiquinone-deficient mutants (Fig. 8 and our unpublished results), but to a lower level in the cytochrome *c* mutant. This suggests that ubiquinone is more directly involved in sulfide oxidation than cytochrome c. In fact, the enzyme sulfide-ubiquinone reductase (Hmt2) is known to be responsible for both sulfide oxidation and ubiquinone reduction. In the absence of ubiquinone, the enzyme is not functional, and thus, sulfide accumulates to a greater extent than in other respiratory-deficient mutants.

The ubiquinone deficient phenotypes are more pronounced in sulfide productions than those of the *cyc1* mutant. The present study also documents for the first time, differential Cu^{2+} sensitivity between ubiquinone-deficient and cytochrome *c* mutants. This suggests that ubiquinone functions as an anti-oxidant in addition to be a component of the respiratory chain. The observations presented herein have distinguished three related functions of ubiquinone: a component of the electron transfer system, an antioxidant, and an anti-sulfide oxidant.

We observed a novel phenotype in respiration-deficient fission yeast mutants. Namely, they rapidly lose viability during stationary phase and this phenotype is occasionally called chronological life span shortage. Such a phenotype has not been reported in an *S. cerevisiae cyc1* mutant whose major deficiency is the inability to grow on a non-fermentable carbon source. In contrast, an *S. pombe cyc1* mutant has a variety of phenotypes as shown in this study. There is a great difference in the dependence on respiration in these two yeasts. Another example of species-specific roles is that both ubiquinone and menaquinone are required by *E. coli* for growth [19].

Thus, species-specific differences in the functions for ubiquinone (or generally quinones) must always be taken into account.

S. pombe is considered a petite negative and *S. cerevisiae* a petite positive yeast. "Petite negative" has been defined as the inability (or near-inability) to lose mitochondrial DNA. One reason why *S. pombe* is petite negative may be related to its primarily aerobic metabolism. Although the first respiration-deficient mutant in *S. pombe* was described thirty seven years ago [47], respiration in this species has not been the subject of the same intensive research, for example, that has been on-going in *S. cerevisiae*, and that has led to large body of knowledge in the areas of respiration and energy metabolism [7]. However, we are now aware that significant differences exist in aerobic energy metabolism between these two yeasts, and in some regards, *S. pombe* appears to resemble higher eukaryotes more closely than *S. cerevisiae*. We suggest that the study of ubiquinone biosynthesis and physiology in *S. pombe* provides a very useful system for exploring differences and similarities in aerobic energy generation in eukaryotes.

Experimental procedures

Materials.

Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo Co. Ltd., (Kyoto, Japan) and from New England Biolabs, Japan, Inc. (Tokyo, Japan)

Strains, plasmids and media.

E. coli strains DH5α and DH10B were used for constructing plasmids. Plasmids pBluescript II KS+/-, pT7blue-T (Novagen, Darmstadt, Germany), pREP1 and pREP1-GFPS65A [48] were used as vectors. The *S. pombe* strains used in this study are listed in Table 1. Yeast cells were grown in YE (0.5% yeast extract, 3% glucose) or PM minimal medium with appropriate supplements as described [49]. YEA and PMA are YE and PM containing 75 μg/ml adenine, respectively. When needed, amino acids were

added to a final concentration of 100 μg/ml. Yeast transformations were performed as described [50].

DNA manipulations.

Cloning, restriction enzyme analysis and preparation of plasmid DNA were performed essentially as described [51]. PCRs were performed as described [52]. DNA sequences were determined by the dideoxynucleotide chain-termination method using an ABI377 DNA sequencer. To clone the *coq7* gene, the following 3 primers (Table 2) were designed. Two primers, Spcoq7-a and Spcoq7-b, were used to amplify a 2.2kb fragment containing the *coq7* gene and flanking sequences. The amplified fragment was then cloned into pBluescript II KS to yield pBPC7. To construct pBUM7, pBPC7 was digested with *Nde*I and ligated with the *ura4* cassette derived from pHSG398-*ura4* [53]. The two primers, Spcoq7-c and Spcoq7-b, were used to amplify the *coq7* gene and the amplified fragment was then cloned into pT7Blue-T to yield pTPC7. The *Sal*I-*Sma*I fragment containing the *coq7* gene was cloned into the *Sal*I-*Sma*I site of pREP1 to yield pREP1-coq7Sp. To clone the *S. cerevisiae COQ7* gene, Sc-Coq7a and Sc-Coq7b were used to generate a fragment that was cloned into pT7Blue-T. To construct pREP1-COQ7, the *Sal*I-*Sma*I fragment was cloned into pREP1. A *Hin*dIII-*Sma*I fragment was cloned into pBSSK-TP45 containing mitochondrial transit sequences for *ppt1* in the *Sal*I-*Hin*dIII site of pBluescript II KS+ [14]. To construct pREP1-TPCOQ7, the *Sal*I-*Sma*I fragment was cloned into pREP1.

Gene disruptions.

The one-step gene disruption technique was performed as described [54]. Plasmid pBUM7 was linearized by appropriate digestions and used to transform SP870 [55] and SP826 [56] to uracil prototrophy. About 200 Ura^+ transformants were picked and grown on YEA-rich medium. The stability of the Ura^+ phenotype was examined by replica plating, and four stable Ura^+ transformants were obtained. One of these strains, designated SP826Δ*coq7*, was sporulated. Germinated haploid cells were replica-plated to plates containing YEA and PMA-Leu. While all cells grew well on YEA medium, some

grew only very slowly on the PMA-Leu plate. One such haploid strain, designated LN902, was used for further experiments. Southern hybridization was performed to confirm integrations as described [51]. The DNA was first digested with *Eco*RV and run on an agarose gel. The *ura4* cassette and the *coq7* gene were then used as probes. In lanes containing LM902 DNA, 6.9- and 5.1-kb bands appeared with both probes (Fig. 2C, lane 2 and 4), as expected because LN902 contains the *ura4*-disrupted *coq7* gene. When the *ura4* cassette was used as probe, no band appeared with DNA from SP870 (Fig. 2C, lane 1). When the *coq7* fragment was used as a probe, a 10-kb band appeared with the SP870 DNA (Fig. 2C, lane3). Thus, it was concluded that the *coq7* gene was disrupted in LN902. *cyc1, coq3 and coq7* disruptants were constructed with a kanamycin marker replacing the coding sequences using the *KanMX6* module [57]. The deletion cassette was constructed using a recombinant PCR approach. DNA fragments of 400-500 bp and corresponding to the 5' and 3' regions of the *coq7 (coq3* or *cyc1)* gene were amplified by PCR using oligonucleotide pairs coq7-w, (coq3-w or cyc1-w) and coq7-x (coq3-x or cyc1-x), and coq7-y (coq3-y or cyc1-y) and coq7-z (coq3-z or cyc1-z), respectively (Table 2). Both amplified fragments were fused to the ends of the *kanMX6* module [35] by PCR. PR110 was transformed with the resulting *coq7::kanMX6 coq3::kanMX6* and *cyc1::kanMX6* fragments. Transformants were selected with G418 (Sigma Chemical Co.). PCR and Southern blot analysis were used to confirm that the chromosomal *coq7, coq3* and *cyc1* genes were properly replaced. The resulting disruptants were designated RM1, RM2 and RM3, respectively.

Ubiquinone extraction and measurement.

Ubiquinone was extracted as described [15]. The crude extract was analyzed by normal-phase TLC with authentic ubiquinone-10 as a standard. Normal-phase TLC was carried out on Kieselgel 60 F_{254} with benzene. The band containing ubiquinone was collected from the TLC plate following UV visualization and extracted with isopropanol/hexane (1:1, v/v). Samples were dried and redissolved in ethanol. The purified ubiquinone was further analyzed by HPLC using ethanol as a solvent.

Mass spectrometry.

Quinone compounds from wild type fission yeast and the *coq7* deletion mutant were purified by HPLC as above. About 1-3 L yeast cultures were used for purification of the quinones. Mass spectra were obtained on a Hitachi M-80 B double-focusing mass spectrometer in the electron impact (EI) mode.

Measurement of sulfide.

Hydrogen sulfide was first detected by production of PbS from lead acetate. Quantitative determination of sulfide was performed by the methylene blue method as described [5]. Briefly, *S. pombe* cells were grown in YEA medium (50 ml) to the time point indicated in Fig. 8. The cells were precipitated by centrifugation and 500 μl of supernatant were mixed with 0.1 ml of 0.1% dimethylphenylenediamine (in 5.5N HCl) and 0.1ml of 23 mM $FeCl_3$ (in 1.2N HCl). The samples were incubated at 37°C for 5 min, after which the absorbance at 670 nm was determined using a blank consisting of the reagents alone.

Oxygen consumption.

Oxygen consumption was measured using an YSI model 53 oxygen monitor.

Staining of mitochondria and fluorescence microscopy.

Mitochondria were stained by the mitochondria-specific dye, MitoTracker Red FM (Molecular Probes, Inc. OR, USA). Cells were suspended in 10 mM HEPES, pH 7.4, containing 5% glucose and MitoTracker Red FM at a final concentration of 100 nM. After 15 min incubation at room temperature, cells were visualized by fluorescence microscopy at 490 nm. Fluorescence microscopy was carried out with a BX51 microscope (Olympus, Corp., Tokyo, Japan) at 1,000X magnification. GFPS65A fluorescence was observed by illumination at 485 nm. Images were captured by a adigital camera DP70 (Olympus, Corp., Tokyo, Japan).

Cell extracts and western blotting.

About 10^8 *S. pombe* cells were harvested. Pellets were washed with STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN_3 [pH8.0]) and stored at -80°C. The

pellets were diluted in 100 μl of dH_2O and boiled at 95℃ for 5 min, after which 120 μl of 2X Laemmli buffer (4% SDS, 20% glycerol, 0.6M ß-mercaptoethanol, 0.12M Tris-HCl [pH6.8]) containing 8M Urea and 0.02% BPB were added to the samples, which were vigorously vortexed with an equal volume of zirconia-silica beads for 3 min and then heated again at 95℃ for 5 min. The zirconia-silica beads and insoluble cell debris were then removed by centrifugation at 10,000 x *g* for 15 min. Approximately equal amounts of each sample were analyzed by SDS-polyacrylamide gel electrophoresis using a 10~15% polyacrylamide gel and then transferred to Immobilon transfer membranes (Millipore, Tokyo, Japan) by a wet-type transfer system. For detection of activated Spc1, membranes were incubated with an anti-p38 MAPK Tyr182 antibody diluted 1:500 in an ECL blocking reagent (GE Healthcare UK Ltd., England), washed, and then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody diluted 1:1000 in an ECL blocking reagent. The secondary antibodies were detected with ECL Advance system as described by the manufacturer (GE Healthcare UK Ltd., England). For detection of Cdc2p, membranes were incubated with an anti-PSTAIRE polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA) diluted 1:1000 in an ECL blocking reagent, washed, and then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody diluted 1:2000 in an ECL blocking reagent. The secondary antibodies were detected with the ECL system (GE Healthcare UK Ltd., England).

RNA preparation and northern blot analysis.

Total RNA from *S. pombe* cells was prepared as follows. About 10^8 cells grown in an appropriate medium were washed with dH_2O, resuspended in 0.5 ml of ISOGEN RNA isolation reagent (Nippon gene, Tokyo, Japan) and vigorously vortexed with an equal volume of zirconia-silica beads for 5 min. Following centrifugation at 10,000 x *g* for 15 min, nucleic acids in the supernatant were precipitated with isopropanol. The RNA was resolved on formaldehyde-agarose gels and transferred to a membrane (Hybond N^+). PCR fragments for *ctt1, gpx1* and *apt1* were used as probes. The probe was labeled

with [α-^{32}P] dCTP (GE Healthcare UK Ltd., England) using a BcaBEST labeling kit (Takara Co. Ltd., Kyoto, Japan).

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FIGURE LEGENDS

Fig. 1. Comparison of amino acid sequences of Clk-1/Coq7p homologs. Alignment of the COQ7 and its orthologous amino acid sequences from *S. pombe* (AL031854), *S. cerevisiae* (X82930), *C. elegans* (U13642), mouse (AF053770), and human (U81276) using the CLUSTAL W method. Conserved amino acid residues are shown in black boxes. Gaps (-) were introduced to maximize the alignment.

Fig. 2. Construction of plasmids and strains. (A) Asterisks indicate the sites of TA ligation with the T-tailed vector pT7Blue-T. pREP1-coq7Sp contains the entire length of the *coq7* gene, and pREP1-TPCOQ7 contains the Ppt1 mitochondrial transit peptide (TP) fused to the N-terminus of the complete *COQ7* gene. Both genes are under the control of the strong *nmt1* promoter. Abbreviations for restriction enzymes are: H, *Hin*dIII; EI, *Eco* RI; N, *Nde* I; Sm, *Sma* I; Sa, *Sal* I; EV, *Eco* RV. (B) The *Eco*RV restriction map of wild type and the *coq7*-disrupted chromosome. (C) Genomic DNA from SP870 and LN902 was prepared, digested with *Eco* RV, and separated on an agarose gel. The *ura4* cassette (a) and the *coq7* gene (b) were used as probes. Lanes 1 and 3, Wild-type SP870; lanes 2 and 4, LN902 (*coq7::ura4*). (D) Schematic depiction of *coq7, coq3* and *cyc1* deletion strains.

Fig. 3. Analysis of ubiquinone and DMQ. (A) Ubiquinone extracted from the LN902 strain (*coq7::ura4*) was first separated by TLC and further analyzed by HPLC. Authentic Q-10 was mixed with the extract from LN902. (B) Mass spectrum of the quinone-like compound from a Δ*coq7* strain. Mass spectrometric analysis indicated that the quinone-like compound yielded an ion with the theoretically calculated mass for protonated DMQ-10 (demethoxy ubiquinone-10).

Fig. 4. Complementation of LN902 (*coq7::ura4*) mutant by *ScCOQ7.* (A) Strain LN902 (*coq7::ura4*) harboring pREP1-TPCOQ7 or pREP1-coq7, and SP66 harboring pREP1 were grown on PM medium containing cysteine (a) or not (b) and their growth was compared. (B) Ubiquinone was extracted from the same strains.

Fig. 5. Sensitivity of LN902 to oxygen radical producers. Wild-type (Square), RM1(Diamond), RM2 (Circle), and RM3 (Triangle) were pre-grown in YEA liquid medium to saturation. Cells were then diluted 40-fold into fresh YEA medium or fresh medium containing 0.5 mM H_2O_2 or 1.5 mM Cu^{2+}. Cell growth was measured at 4-h intervals using a cell counter (Sysmex Corp.).

Fig. 6. Northern analysis of stress-responsive genes. (A) Wild-type SP870, and RM19 (*Δdlp1*), KS10 (*Δdps1*), RM3 (*Δcyc1*), LN902 (*Δcoq7*), NBp17 (*Δcoq8*), and TK105 (*Δspc1*) were used. Total RNAs were isolated from mid-log cultures of the indicated strains and from SP870 treated with 1 mM H_2O_2 for 15 min. RNAs were separated by electrophoresis and northern blots were then probed sequentially using DNA specific for the *ctt1*$^+$, *gpx1*$^+$ and *apt1*$^+$ genes. *leu1*$^+$ mRNA was used as a loading control. (B) The level of expression detected in (A) was standardized by NIH image. Lane 1, wild-type; lane 2, wild type with 1 mM H_2O_2 for 15 min; lane 3,*Δ dlp1*; lane 4, Δ*dps1*; lane 5, Δ*cyc1*; lane 6,Δ*coq7*; lane 7, *Δcoq8*; lane 8, Δ*spc1*.

Fig. 7. Western blot analysis. PR110, and RM1 (*Δcoq7*), RM2 (*Δcoq3*), RM3(*Δcyc1*), JV5(*Δhmt2*) , and DS31 (*Δsir1*) were grown in YEA liquid medium at 30°C and cells were grown to 0.5x10^7 cells/ml. PR110 was treated with 1 mM H_2O_2. Crude protein extracts of the indicated cells were prepared by boiling. Western blotting was performed using anti-p38 and an anti-PSTAIRE antibody as a loading control.

Fig. 8. Sulfide production. PR110, and RM1 (*Δcoq7*), RM2 (*Δcoq3*), RM3(*Δcyc1*),

JV5(*Δhmt2*) , and DS31 (*Δsir1*) were grown in YEA medium. The amount of sulfide produced was measured by the methylene blue method at 4 hr intervals.

Fig. 9. Loss of viability during stationary phase. PR110 and RM1 (*Δcoq7*), RM2 (*Δcoq3*), RM3 (*Δcyc1*), and JZ858 (*Δcgs1*) were pre-grown in YEA liquid medium at 30 °C and then grown in PMALU supplemented with cysteine. When the cells reached 1.0 x10^7 cells/ml, viability was measured by plating on YEA plates after appropriate dilution.

Fig. 10. Co-localization of Coq7-GFP fusion proteins with a mitochondrion-specific dye. Phase contrast images of cells, GFP fluorescence produced by Coq7-GFP fusion proteins, and mitochondrial staining by Mito Tracker in strain LN902 expressing Coq7-GFP are shown.

Table 1. *S. pombe* strains used in this study.

Strain	Genotype	Source
SP826	*h⁺ ade6-210 leu1-32 ura4-D18/ h⁺ ade6-216 leu1-32 ura4-D18*	Lab stock
SP870	*h⁹⁰ ade6-210 leu1-32 ura4-D18*	Lab stock
PR110	*h⁺ leu1-32 ura4-D18*	Lab stock
KS10	*h⁺ ade6-210 leu1-32 ura4-D18 dps1::ura4*	(3)
RM19	*h⁺ leu1-32 ura4-D18 dlp1:: kanMX6*	(5)
DS31	*h⁹⁰ leu1-32 ura4-294 sir1::LEU2*	(4)
JV5	*h⁻ leu1-32 ura4-294 hmt2::URA3*	(4)
TK105	*h⁹⁰ leu1-32 ura4-D18 spc1::ura4*	Katoh
JZ858	*h⁹⁰ ade6-216 leu1-32 ura4-D18 cgs1::ura4*	Yamamoto
LN902	*h⁹⁰ ade6-210 leu1-32 ura4-D18 coq7::ura4*	This study
RM1	*h⁺ leu1-32 ura4-D18 coq7::kanMX6*	This study
RM2	*h⁺ leu1-32 ura4-D18 coq3::kanMX6*	This study
RM3	*h⁺ leu1-32 ura4-D18 cyc1::kanMX6*	This study

Table 2. Oligonucleotide primers used in this study.

ScCoq7-a	5'-CCGTCGACCAAGCTTATGTTTCCTTATTTTTACAGACG-3'
ScCoq7-b	5'-CCCCCGGGGCCACTTTCTGGTG-3'
Spcoq7-a	5'-GTACAAGCTTGTAAATTTTCGATGG-3'
Spcoq7-b	5'-CATAGAATTCTTGGTAATC -3'
Spcoq7-c	5'-AAAGTCGACATGTTGTCACGTAGACAG-3'
Spcoq7-w	5'-CAAGCAGGTGAATTAGGC-3'
Spcoq7-x	5'-GGGGATCCGTCGACCTGCAGCGTACGAAAATCGTTTACACATC-3'
Spcoq7-y	5'-GTTTAAACGAGCTCGAATTCATCGATGCTAGTCCTTTATG-3'
Spcoq7-z	5'-CAGGCAAGTCTGTTTATTG-3'
Spcoq7-m	5'-CTTGGATGAGCTTTCCAC-3'
Spcoq3-w	5'-CGTATAAATTACAATACCG-3'
Spcoq3-x	5'-GGGGATCCGTCGACCTGCAGCGTACGACATACTACTTCATTTG-3'
Spcoq3-y	5'-GTTTAAACGAGCTCGAATTCATCGATCCTAGCGTTACCGTTG-3'
Spcoq3-z	5'-GTATGCGATGTGGAATTTG-3'
Spcoq3-m	5'-GATGCCTTCCAATGAATTAC-3'
cyc1-w	5'-GAACCAATGAAATAAGGGCG-3'
cyc1-x	5'-GGGGATCCGTCGACCTGCAGCGTACGAGGAAAGGAAATAGGC-3'
cyc1-y	5'-GTTTAAACGAGCTCGAATTCATCGATCCGTCAACGACAGTTG-3'
cyc1-z	5'-GCATCAGAAAGCATAGGC-3'
cyc1-m	5'-TGGGAATACGATAGAGTAG-3'
nb2 primer	5'-GTTTAAACGAGCTCGAATTC-3'

```
hCOQ7                                                          MSCAGAAA   8
mCOQ7                                                          MSAAGAIA   8
ScCoq7p    MFPYFYRREF YSCENVVIFS SKPIQGIKIS RIRERYIEIM LSRVSVFKPA  50
SpCoq7p                                                  M LSRRQLIPIA  11

hCOQ7      APRLWRLRPG ARRSLSAYGR RTSVRFRSSG MTLDNISRAA VDRIIRVDHA  58
mCOQ7      AASVGRLRTG VRRPFSEYGR GLIIRCHSSG MTLDNINRAA VDRIIRVDHA  58
CeClk-1                          MFRVITRG AHTAASRQAL IEKIIRVDHA  28
ScCoq7p    SRGFSVLSSL KITEHTSAKH TEKPEHAPKC QNLSDAQAAF LDRVIRVDQA 100
SpCoq7p    .........T KSVSPFLRSS ITCRRISMGR KELSEKDSNI LDSVIRVDQA  52

hCOQ7      GEYGANRIYA GQMAVLGRTS ..VGPVIQKM WDQEKDHLKK FNELMVMFRV 106
mCOQ7      GEYGANRIYA GQMAVLGRTS ..VGPVIQKM WDQEKNHLKK FNELMIAFRV 106
CeClk-1    GELGADRIYA GQLAVLQGSS ..VGSVIKKM WDEEKEHLDT MERLAAKHNV  76
ScCoq7p    GELGADYIYA GQYFVLAHRY PHLKPVLKHI WDQEIHHHNT FNNLQLKRRV 150
SpCoq7p    GELGANQIYK GQHFILQFTD PKVAPTIQHM WDQEKYHLAT FDNYVLKNRV 102

hCOQ7      RPTVLMPLWN VLGFALGAGT ALLGKEGAMA CTVAVEESIA HHYNNQIR.. 154
mCOQ7      RPTVLMPLWN VAGFALGAGT ALLGKEGAMA CTVAVEESIA NHYNNQIR.. 154
CeClk-1    PHTVFSPVFS VAAYALGVGS ALLGKEGAMA CTIAVEELIG QHYNDQLK.. 124
ScCoq7p    RPSLLTPLWK AGAFAMGAGT ALISPEAAMA CTEAVETVIG GHYNGQLRNL 200
SpCoq7p    RPTFLRPFWD IAGFALGAGT ALLGTKAAMA CTEAVETVIG GHYNDQLR.. 150

hCOQ7      .........T LMEEDPEKYE ELLQLIKKFR DEELEHHDIG LD.HDAELAP 194
mCOQ7      .........M LMEEDPEKYE ELLQVIKQFR DEELEHHDTG LD.HDAELAP 194
CeClk-1    .........E LLADDPETHK ELLKILTRLR DEELHHHDTG VE.HDGMKAP 164
ScCoq7p    ANQFNLERTD GTKGPSEEIK SLTSTIQQFR DDELEHLDTA IK.HDSYMAV 249
SpCoq7p    .......ETA HLENKAPEFK EIRSHLAEFR DDELEHLNTA VEGWNAKEAP 193

hCOQ7      AYAVLKSIIQ AGCRVAIYLS ERL 217
mCOQ7      AYALLKRIIQ AGCSAAIYLS ERF 217
CeClk-1    AYSALKWIIQ TGCKGAIAIA EKI 187
ScCoq7p    PYTVITEGIK TICRVAIWSA ERI 272
SpCoq7p    AHALLTNAIQ MGCKAAIWMC KRF 216
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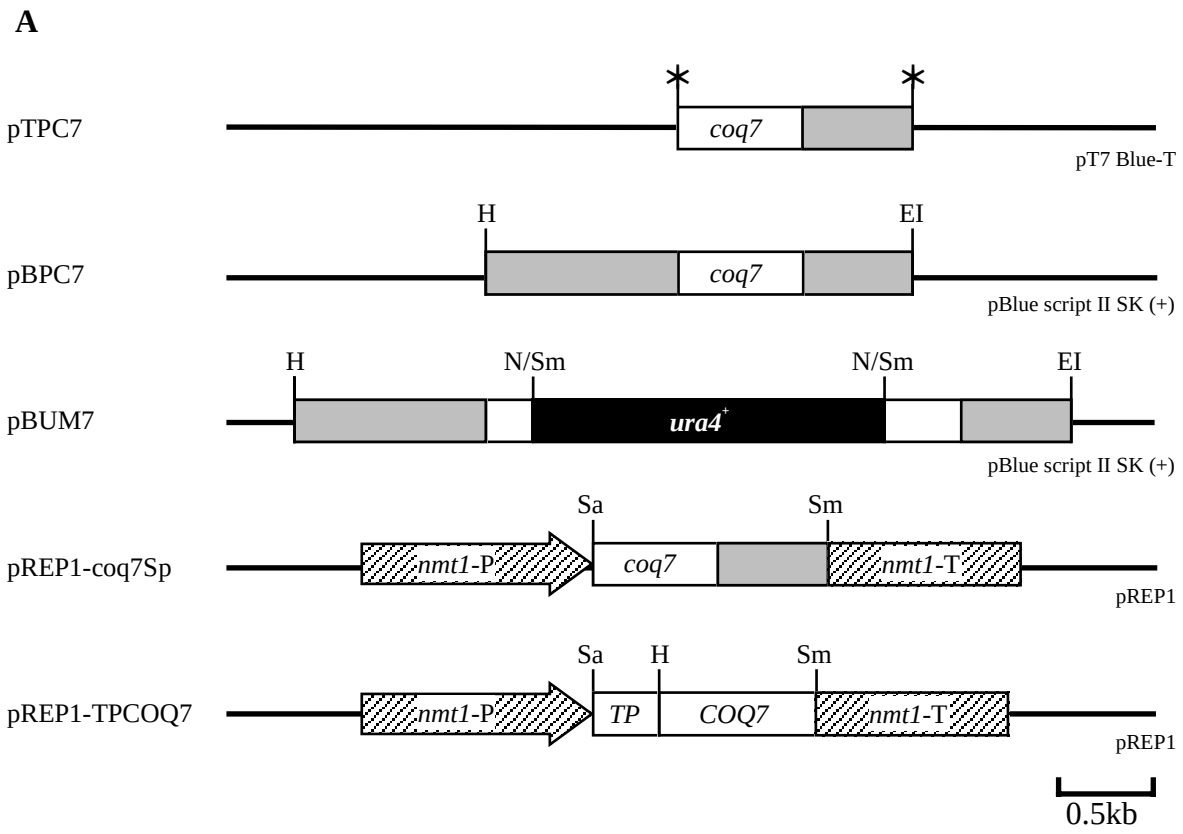
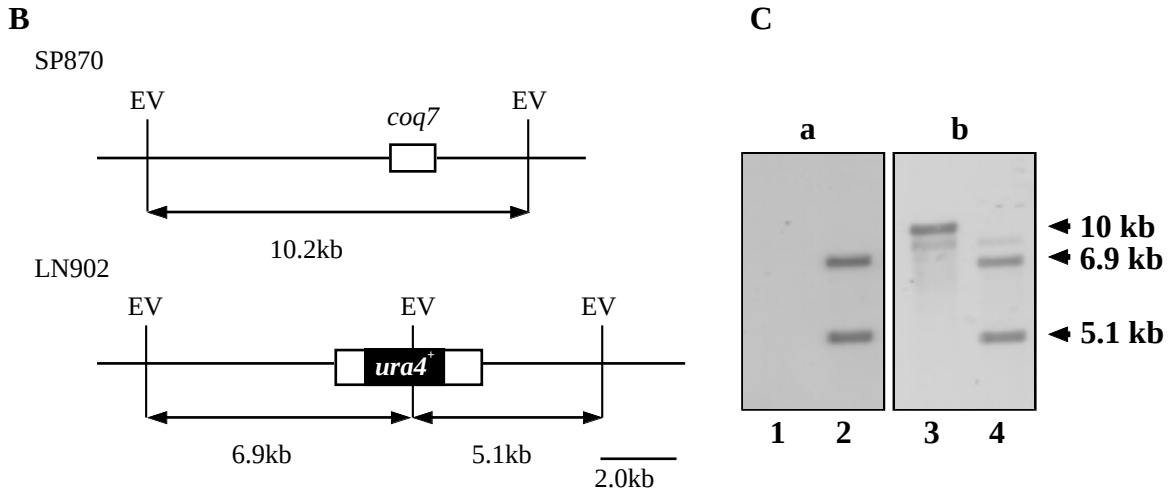

A
pTPC7
coq7
pT7 Blue-T
H
EI
pBPC7
coq7
pBlue script II SK (+)
H
N/Sm
N/Sm
EI
pBUM7
ura4+
pBlue script II SK (+)
Sa
Sm
pREP1-coq7Sp
nmt1-P
coq7
nmt1-T
pREP1
Sa
H
Sm
pREP1-TPCOQ7
nmt1-P
TP
COQ7
nmt1-T
pREP1
0.5kb


B
SP870
EV
coq7
EV
10.2kb
LN902
EV
EV
EV
ura4+
6.9kb
5.1kb
2.0kb
C
a
b
10 kb
6.9 kb
5.1 kb
1
2
3
4

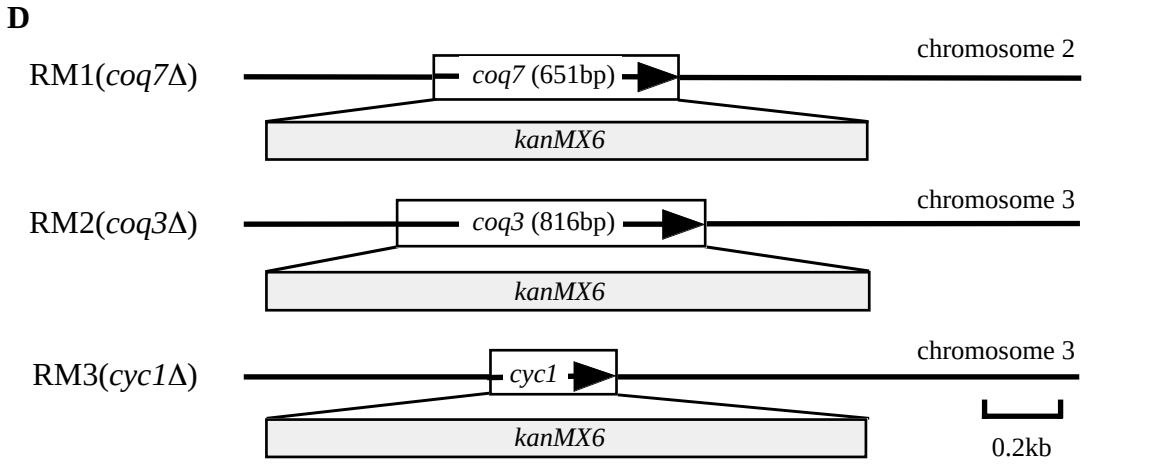
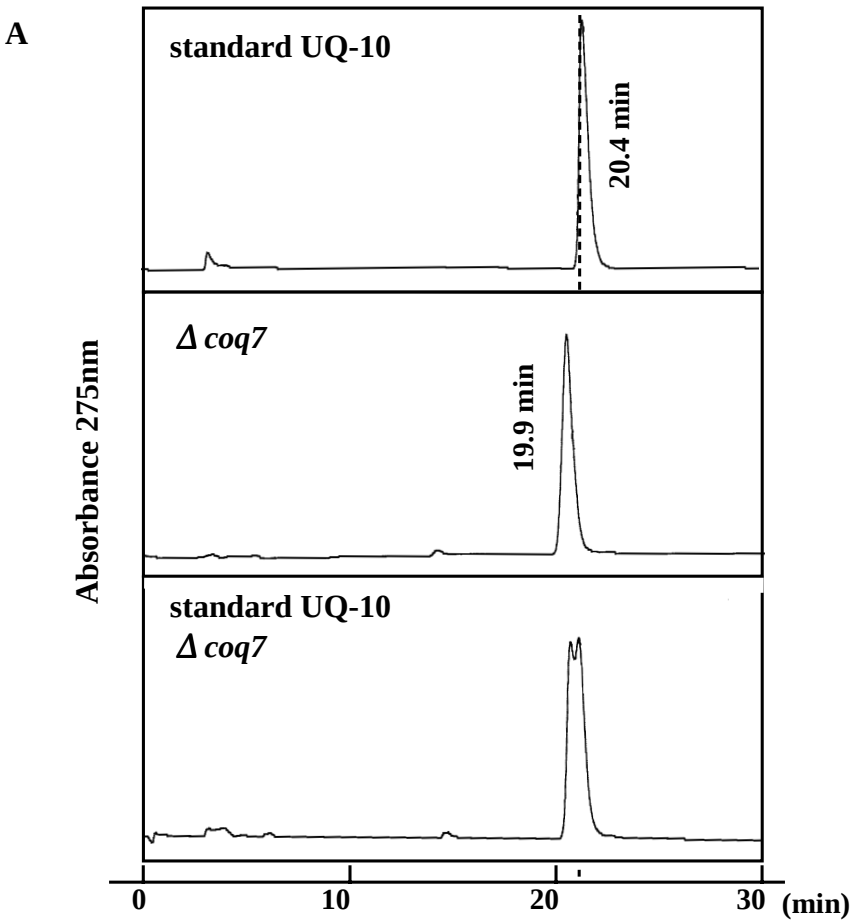

D
RM1(coq7Δ)
coq7 (651bp)
kanMX6
chromosome 2
RM2(coq3Δ)
coq3 (816bp)
kanMX6
chromosome 3
RM3(cyc1Δ)
cyc1
kanMX6
chromosome 3
0.2kb

Fig. 2


A
standard UQ-10
20.4 min
Δ coq7
19.9 min
standard UQ-10
Δ coq7
Absorbance 275nm
0
10
20
30
(min)

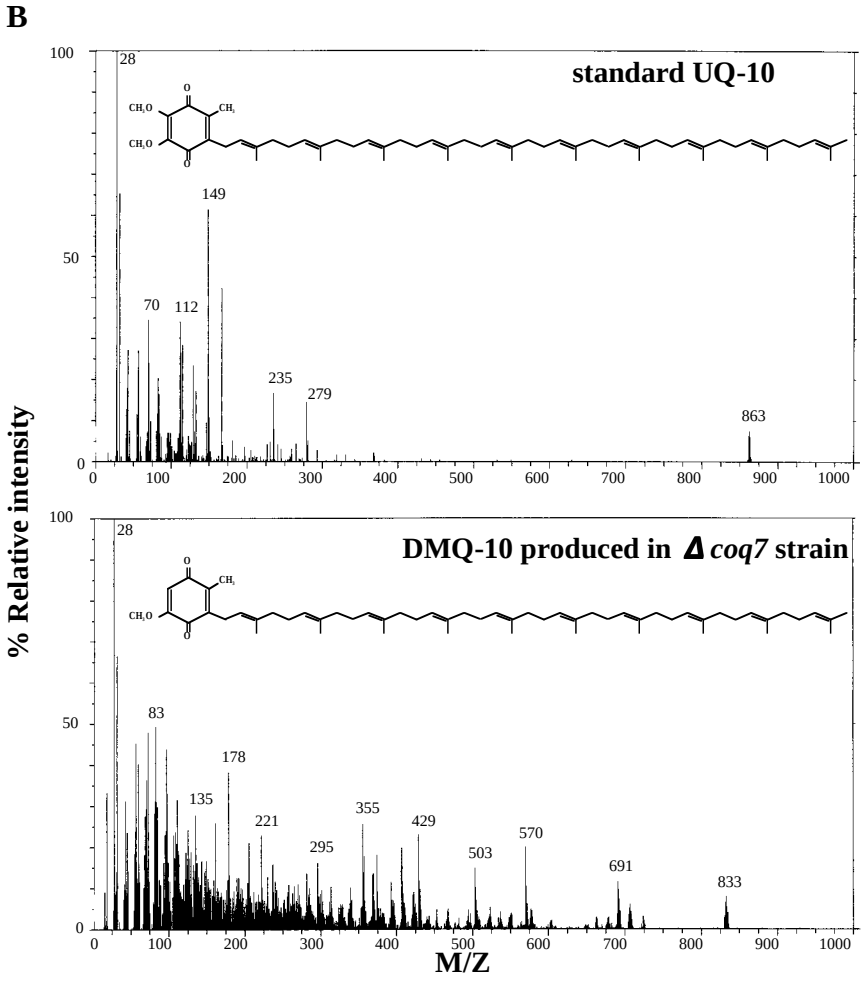
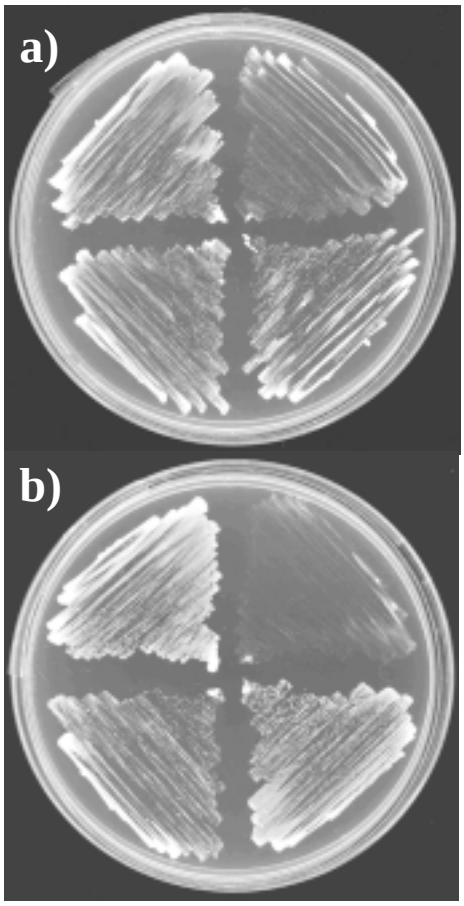
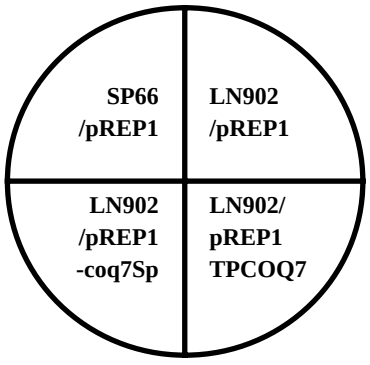

B
standard UQ-10
28
149
70
112
235
279
863
DMQ-10 produced in Δ coq7 strain
28
83
178
135
221
295
355
429
503
570
691
833
% Relative intensity
M/Z

Fig. 3

A


a)
b)


SP66
/pREP1
LN902
/pREP1
LN902
/pREP1
-coq7Sp
LN902/
pREP1
TPCOQ7

B

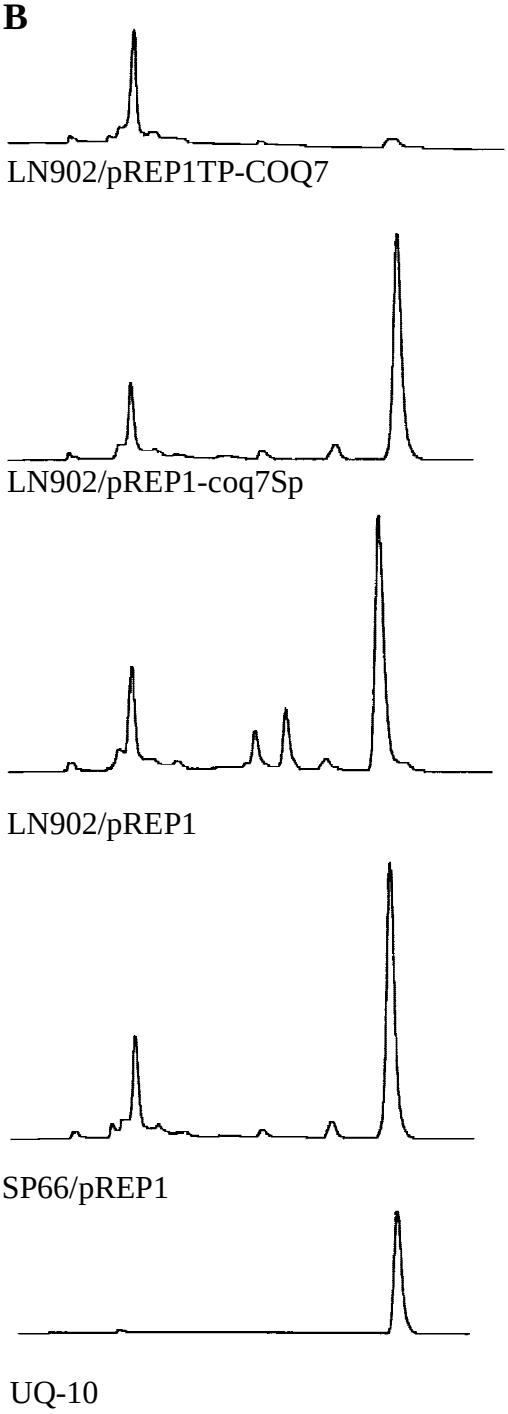

LN902/pREP1TP-COQ7
LN902/pREP1-coq7Sp
LN902/pREP1
SP66/pREP1
UQ-10

Fig. 4

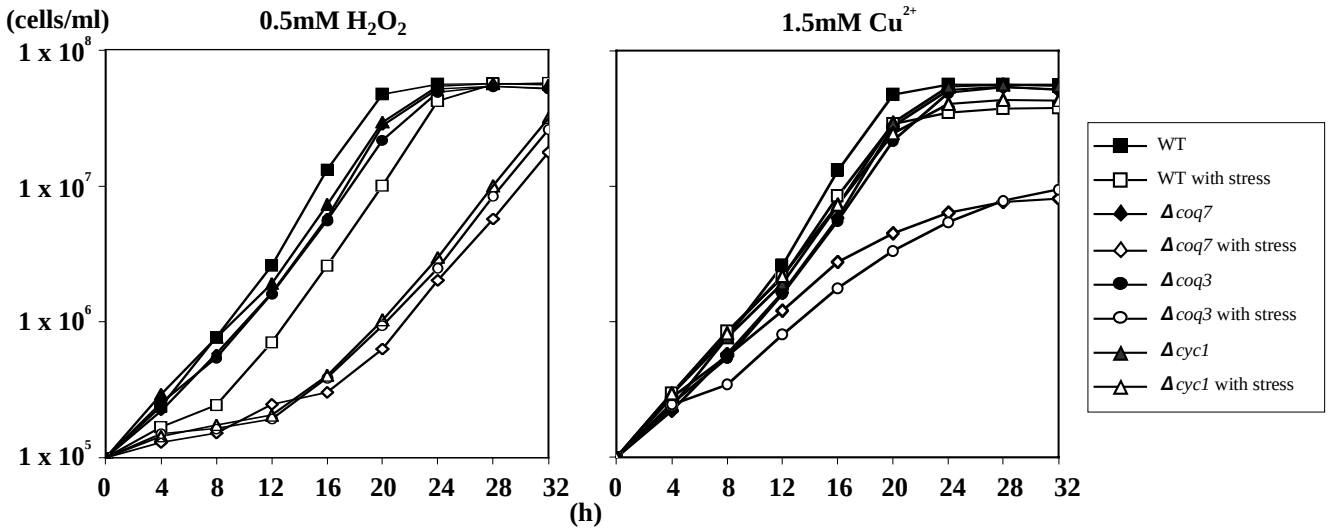
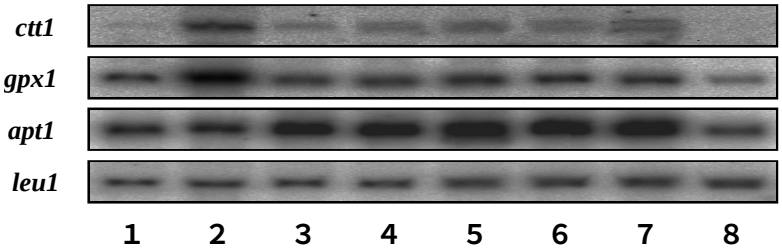

(cells/ml)
0.5mM H_2O_2
1.5mM Cu^{2+}
1 x 10^8
1 x 10^7
1 x 10^6
1 x 10^5
0 4 8 12 16 20 24 28 32
0 4 8 12 16 20 24 28 32
(h)
WT
WT with stress
Δcoq7
Δcoq7 with stress
Δcoq3
Δcoq3 with stress
Δcyc1
Δcyc1 with stress

Fig. 5

A


ctt1
gpx1
apt1
leu1
1 2 3 4 5 6 7 8

B

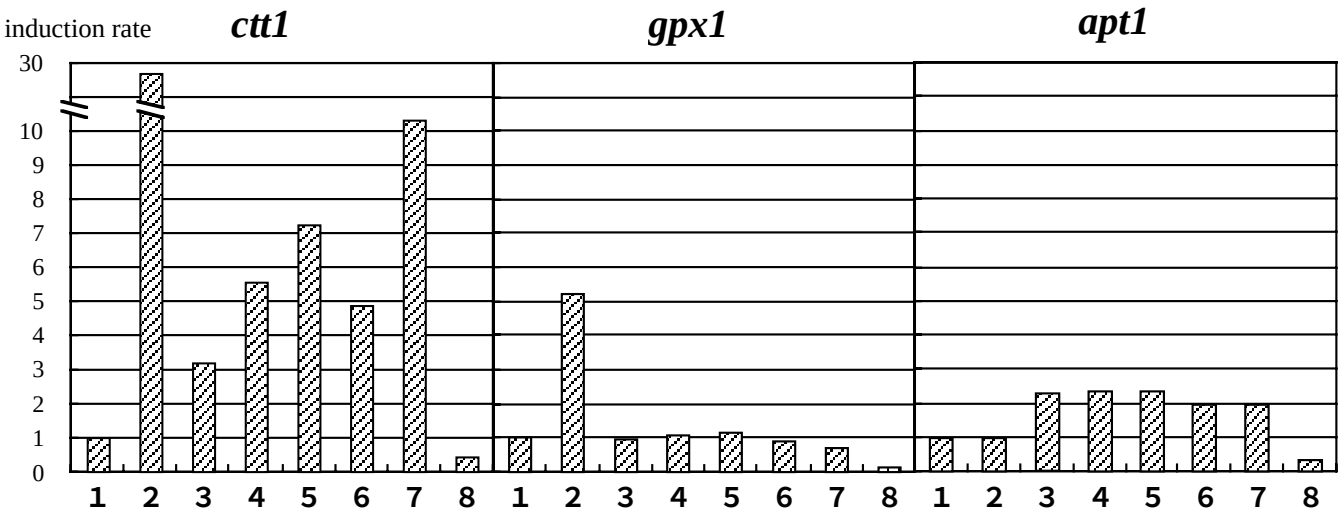

induction rate
ctt1
gpx1
apt1
30
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8

Fig. 6

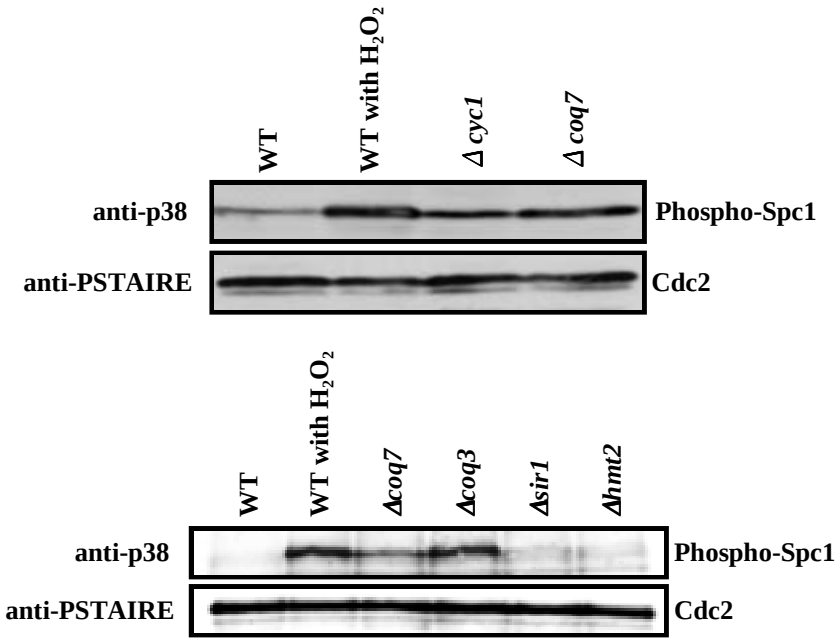

WT
WT with H_2O_2
Δ cyc1
Δ coq7
anti-p38
Phospho-Spc1
anti-PSTAIRE
Cdc2
WT
WT with H_2O_2
Δcoq7
Δcoq3
Δsir1
Δhmt2
anti-p38
Phospho-Spc1
anti-PSTAIRE
Cdc2

Fig. 7

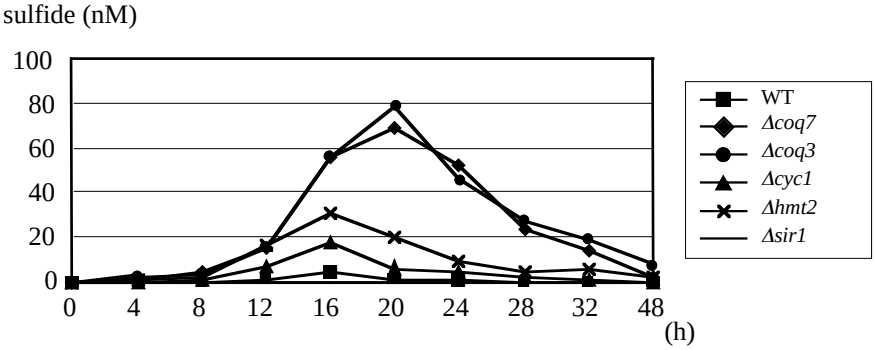

sulfide (nM)
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
48
(h)
WT
Δcoq7
Δcoq3
Δcyc1
Δhmt2
Δsir1

Fig. 8

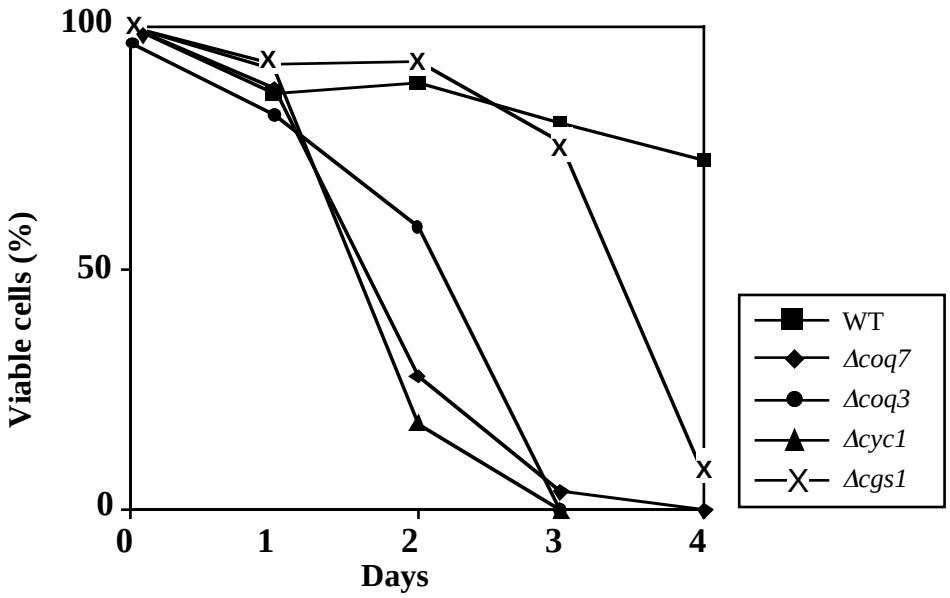
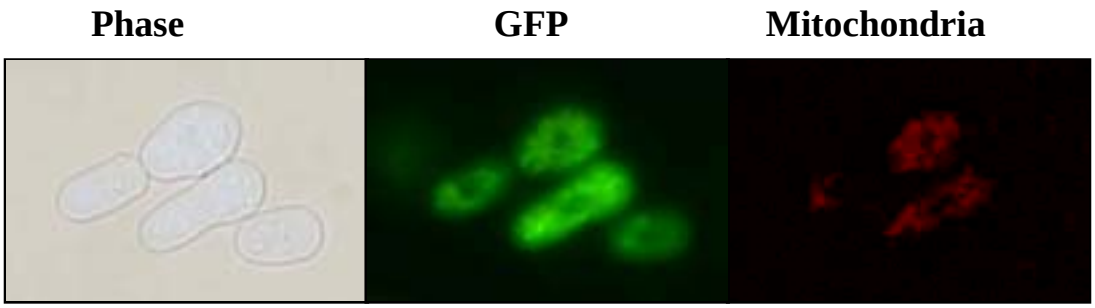


Fig. 9



LN902/pCoq7Sp-GFP

Fig. 10